SHIMADZU

Application News

High Performance Liquid Chromatography SSI LC 004

Rapid Analysis of Polycyclic Aromatic Hydrocarbons in Seafood using Shimadzu Nexera UHPLC with Fluorescence Detection

Introduction

The recent Deepwater Horizon oil spill in the Gulf of Mexico created concerns over the safety of seafood that can get potentially contaminated with Polycyclic Aromatic Hydrocarbons (PAHs) from crude oil. The US Environmental Protection Agency identifies PAHs as critical pollutants harmful to human health. Some of these compounds are known carcinogens. Recently, some Gulf jurisdictions started screening seafood for at least 12 of the PAHs. The method commonly employed for the analysis of PAHs is GCMS, which is quite time consuming and takes upward of 60 minutes, as the GC columns required for PAHs are normally 30 m long with an inner diameter of 0.25 mm and 0.25 µm film thickness. An alternative to GCMS is ultra-high speed LC using fluorescence detection. In this Application News we present a rapid analysis method for Polycyclic Aromatic Hydrocarbons in fish and mollusks using the Shimadzu all-round Nexera UHPLC equipped with a sub 2-micron PAH column and the new RF-20Axs fluorescence detector that offers world-leading levels of sensitivity. A water Raman S/N ratio of this detector is at least 2,000, making it a powerful tool for analyses that demand the detection of trace-level components. When using dark signal as noise reference, however, a water Raman S/N ratio is at least 20,000. The RF-20Axs also features a temperature-controlled cell with a cooling function, maintaining a constant detector cell temperature, even if the room temperature fluctuates significantly, to ensure superb reproducibility with no drop in sensitivity. The 100 Hz sampling rate permits ultra fast LC analysis with no loss of separation. The described method allows for accurate determination of 15 PAHs in less than 4 minutes.

Sample Preparation

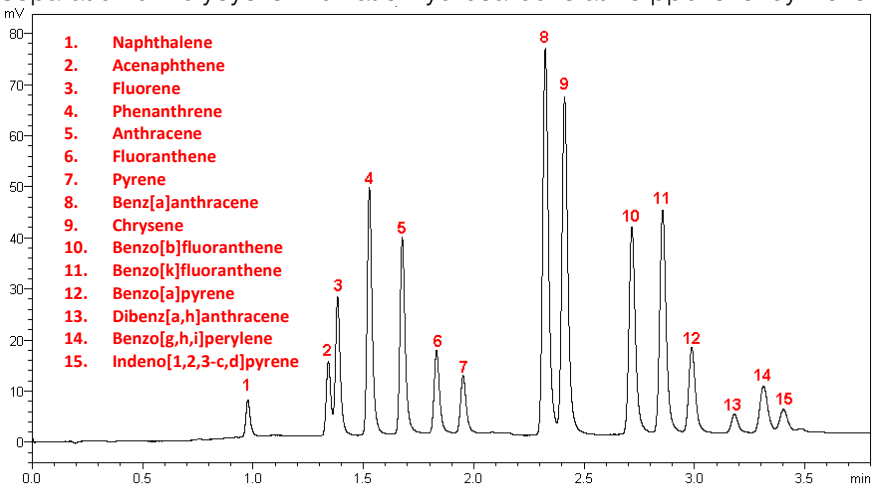
To prepare the sample for injection into the chromatography system a simple QuEChERS method was used. The technique is based on sample cleanup by Dispersive Solid Phase Extraction (dSPE) preceded by an extraction step of a homogenized tissue sample. The following steps are performed by an analyst:

1. Homogenize the tissue sample to uniformity.
2. Weigh 2 g of the homogenate into a clean 15 mL tube.
3. Add 2 mL of acetonitrile and shake for 1 minute (internal standards are added at this step).
4. Add 1.3 g of Resprep™ Q110 (Restek Corporation) and shake for 1 minute.
5. Centrifuge for 5 minutes at 3,000 rpm.
6. Transfer 0.5 ml of the supernatant into an empty dSPE tube.
7. Add 100 mg of Resprep™ Q211 (Restek Corporation) and shake for 30 seconds.
8. Centrifuge for 5 minutes at 3,000 rpm.
9. Adjust the pH of the supernatant by adding 5 µL of 5% formic acid in acetonitrile.
10. Transfer sample into an HPLC vial.

It takes one person about 20 minutes to prepare 16 samples allowing for more than 300 samples per day to be prepared by one analyst and loaded into Nexera UHPLC for an overnight run.

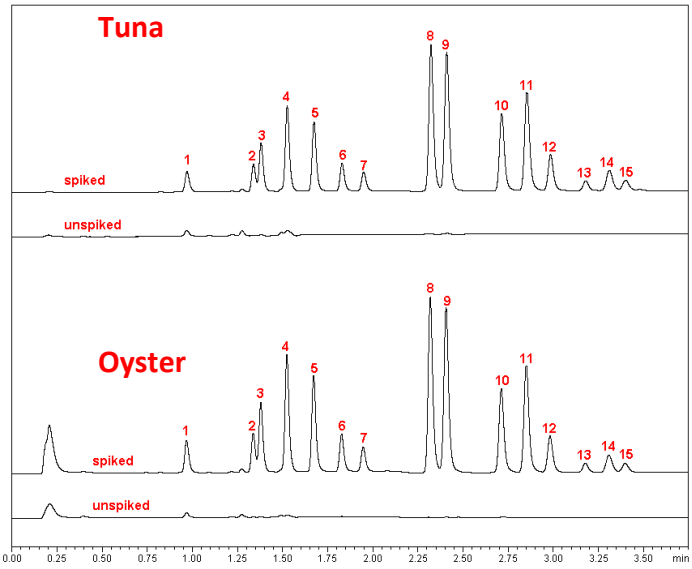
Chromatograms

Separation of Polycyclic Aromatic Hydrocarbons at 20 ppb level by Nexera UHPLC.



Analytical Conditions

- Instrument: Nexera UHPLC with RF-20Axs Detector
- Column: PAH C18 (2.1 x 50 mm, 1.8 µm)
- Mobile Phase: Acetonitrile in Water
- Gradient: Acetonitrile 50% (0.20 min)→100% (2.80 min)
  Acetonitrile 100% (3.50 min)→50% (3.51 min)
- Flow: 0.6 mL/min
- Column Temperature: 30 ℃
- Injection Volume: 2 µL
- Detection: 0.00–1.59 min Ex : 260 nm, Em : 350 nm
  1.59–2.10 min Ex : 260 nm, Em : 440 nm
  2.10–2.54 min Ex : 260 nm, Em : 400 nm
  2.54–3.50 min Ex : 280 nm, Em : 470 nm



Samples of tuna and oyster vs. the same samples spiked with PAHs at ppb level.

Reproducibility Data

A 2µL injection of a mix of PAH standards with 200ppb concentration of each PAH.

Standard	Naphthalene (peak #1)		Fluorene (peak #3)		Anthracene (peak #5)		Benzo[b]fluoranthene (peak #10)	
	Ret. Time (min)	Peak Area	Ret. Time (min)	Peak Area	Ret. Time (min)	Peak Area	Ret. Time (min)	Peak Area
Standard 1	0.974	128,724	1.383	456,059	1.676	664,779	2.713	915,153
Standard 2	0.975	129,256	1.383	457,388	1.676	666,079	2.714	921,063
Standard 3	0.977	129,426	1.384	454,798	1.676	665,638	2.714	918,456
Standard 4	0.978	128,928	1.385	456,915	1.678	665,161	2.716	916,861
Standard 5	0.976	129,372	1.384	455,080	1.677	661,160	2.716	919,007
Standard 6	0.978	129,050	1.386	455,087	1.679	663,834	2.718	917,665
%RSD	**0.165**	**0.212**	**0.086**	**0.237**	**0.079**	**0.268**	**0.063**	**0.218**

Shimadzu Scientific Instruments
7102 Riverwood Drive, Columbia, MD 21046
Phone: 800-477-1227, Fax: 410-381-1222
www.ssi.shimadzu.com
webmaster@shimadzu.com